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A COMPARATIVE STUDY OF THE
EFFECT OF CUMARIN AND VANILLIN ON
WHEAT GROWN IN SOIL, SAND, AND
WATER CULTURES

A THESIS

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BY

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A COMPARATIVE STUDY OF THE EFFECT OF CUMARIN AND VANILLIN ON WHEAT GROWN IN SOIL, SAND, AND WATER CULTURES.¹

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(Contribution from the Department of Soil Technology, Cornell University.)

THE PRESENT STATUS OF THE THEORY OF SOIL TOXICITY.

INTRODUCTION.

The theory of soil toxicity dates from the time of De Candolle. Believing that the experiments of Macaire,² which were carried out at his suggestion, proved that plant roots secrete under normal conditions certain organic substances, which in the case of the bean were found to be harmful to the plant that produced them but beneficial to other plants, De Candolle came to consider these root secretions of universal significance in practical agriculture. He proposed to explain the necessity for crop rotation as based on the fact that plants excrete through their roots certain substances that are deleterious to

¹ A thesis submitted to the faculty of the Graduate School of Cornell University in partial fulfillment of the requirements for the degree of Doctor of Philosophy by Jehiel Davidson, B. Sc. Ithaca, N. Y., June, 1914. Received for publication March 30, 1915.

² Macaire, *Memoire pour Servir a L'Histoire des Assolemens*, Ann. de Chim. et Phys., 52 (1833), p. 225-240. De Candolle, A. P., *Physiologie Vegetale*, p. 248-251. Paris, 1832.

the same plants or their near relatives, but which are beneficial to other plants more or less distantly related to them.³

Liebig⁴ considered De Candolle's theory of crop rotations "as resting on a firm basis" and placed full reliance in the experiments of Macaire. Although he interpreted the views of both of them in the light of his favorite plant-food theory, he considered the conversion of the injurious excrements of plants into humus as a matter of great importance to soil fertility.

The experiments of Macaire which constituted the principal evidence in favor of harmful root secretions, as well as of root secretions in general, were proved to be erroneous by Braconnot⁵ and others, and De Candolle's views lost their adherents and were forgotten under the dominance of Liebig's mineral theory.

The theory of soil toxicity has been brought to the front again by the Federal Bureau of Soils. It has been modified and broadened. Harmful root excretions and toxic organic substances in general account, according to their views, not only for the inability of a soil to grow the same crop successively for a number of years, but also for the infertility of poor soils in general. They oppose the theory of soil toxicity to Liebig's mineral theory, which in its principal features still has a hold on the minds of the majority of agricultural investigators. To these investigators, plant food, whether of mineral or of organic origin, whether produced by physical, chemical or biological agencies, whether found in the soil originally or introduced in the form of manures and fertilizers, is still the principal key to soil fertility.

CHARACTER OF THE EVIDENCE IN FAVOR OF THE THEORY OF SOIL TOXICITY.

The investigators in the Bureau of Soils have brought forward a considerable amount of evidence in support of their views. The evidence may be divided in two groups. One follows the trail of De Candolle and deals with root secretions, the other deals with the presence in the soil of toxic organic substances in general. It is all, however, of indirect nature and, like all indirect evidence, it holds good only so long as the phenomena on which it is based can be explained only by the theory which it supports.

³ De Candolle, l. c., pp. 1474-75 and pp. 1493-1520.

⁴ Liebig, Justus, *Chemistry in its Application to Agriculture and Physiology*, p. 163-174. Cambridge, 1842.

⁵ Braconnot, H., *Recherches sur l'Influence des Plantes sur le Sol*, *Ann. de Chim. et Phys.*, 62 (1839), p. 27-40.

Any other explanation which could be offered to account for the phenomena that serve as evidence in favor of the theory of soil toxicity would rob the evidence of its principal force and relegate the theory in question to a mere hypothesis, more or less plausible. It remains to be seen whether the evidence brought forward stands the criterion of indirect evidence, that is, whether the phenomena on which it is based can not be explained in any way except by the theory of soil toxicity.

CROP ROTATIONS.

No new evidence has been brought forward since the time of De Candolle to substantiate the view that the failure to grow one crop successfully year after year is due to autotoxic substances secreted by the plant roots. The experiments of Macaire which formed the principal basis of De Candolle's theory, as stated above, have been found to be entirely erroneous. The experiments at Rothamsted⁶ where wheat was grown successfully for fifty years in succession would tend to serve as evidence against the secretion of autotoxic substances by the roots. Up to the present time no root secretions except carbon dioxide have been definitely established.

Passing from facts to general considerations, we can easily come to the conclusion that autotoxic excreta in plants are inconsistent with the general laws of adaptation. We could conceive of excreta which are harmful to other plants as a weapon in the struggle for survival, as was suggested by Humboldt and Plenk⁷ with reference to the existence of plant associations. It is hard, however, to conceive how an autotoxic excretion helped the plants possessing it to survive in the struggle for existence or at least how it did not interfere with them in this struggle.

As to the explanation of the beneficial effects of crop rotations, there are a great number of other factors besides autotoxic secreta which may account for them. These include the different methods of cultivation associated with the different crops in the rotations, the different methods of feeding, the difference in the microbiological flora which accompanies the different crops, etc.

EFFECT OF GRASS ON TREES.

It has been observed on the Woburn Experimental Fruit Farm that grass was injurious to fruit trees. The effect of the grass was so

⁶ Gilbert, J. H., *Agricultural Investigations at Rothamsted*, U. S. Dept. Agr., Office of Exp. Sta. Bul. 22, p. 146-171.

⁷ De Candolle, l. c., p. 1474-76.

singular in its character that, according to the authors of the reports, every tree which was grassed over could be easily recognized even if the surface soil were entirely hidden from view.

"The coloring matters in the leaves, bark and fruit are affected in a manner which is not produced by any other form of ill treatment. The bark is pale and much yellower than in a healthy tree; the buds burst at a comparatively early date; and the foliage always exhibits a pale sickly hue, which is quite different from that of trees in the open ground. The autumn tints appear some two weeks earlier than in healthy trees. The fruit, when there is any, shows similar peculiarities of coloring; in case of green apples, for instance, the color is changed either to waxy yellow or to brilliant red."⁸

The ill effects of the grass were shown with reference to old trees as well as to newly planted trees. The effects on the latter were in many cases fatal, leading to death after the first season.

The possible reasons for the ill effects of the grass that suggested themselves were deficiency in moisture due to excessive loss of water caused by transpiration from the grass, deficiency in plant nutrients due to competition of the grass, lack of aeration, excessive amounts of carbon dioxide due to respiration of the grass roots, and differences in temperature. The authors of the reports of the Woburn Fruit Farm checked up the influence of every one of these factors and found that none of them could be considered responsible for the characteristic ill effects on the trees produced by the grass. They therefore reached the conclusion that the effect of the grass was due to some direct poisonous action produced by the grass, either through the agency of micro-organisms for the development of which it offers favorable conditions, or directly by secreting some poisonous substance through the roots.

On a closer examination, however, of the methods used in the experiments which were carried out with the object of checking up the individual influences of the factors mentioned above, we find that they were not thorough enough to justify the negative attitude of the experimenters toward these factors with reference to their instrumentality in the ill effects caused by the grass on the apple trees.

To check up the influence of the moisture factor, the experimenters supplied water artificially to the grassed-over trees and found no improvement. They grew trees in closed pots with a supply of moisture limited to such an extent the trees showed signs of actual suffering from thirst, but the peculiar grass effects could not be observed, although the vigor of the trees was markedly impaired. They could

⁸ Pickering, S. P., *The Effects of Grass on Apple Trees*, Jour. Royal Agr. Soc. of England, 64 (1903), p. 373.

not observe any difference in the appearance of the grassed-over trees between dry and wet seasons. The grassed-over trees never showed any indication of actual suffering from thirst.⁹

As the soil on the experimental farm was shallow, there is a question how much and for how long the artificial applications of water which were made weekly and the rains of the wet seasons increased the actual moisture content of the soil. There is also doubt as to how much of the increase was left at the disposal of the trees, taking in consideration the fact that grass is such a powerful competitor for moisture. That the trees in the closed pots did not show the characteristic peculiarities of the grassed-over trees might be due to the fact that the trees in the pots showed actual signs of suffering from thirst, while the water supply of the grassed-over trees was not deficient to that extent. Would it not have been more direct to have grown trees in pots together with grass and to have had the moisture properly controlled by weighings at regular intervals?

In order to check up the aeration factor, the soil under the grassed-over trees was aerated in various ways and the soil under trees grown without grass was prevented from being aerated as effectively as possible. No change was observed by the experimenters in the behavior of the trees under either treatment.

However, no mention is made of how effective the artificial aeration proved to be, that is, whether or not the soil air was actually enriched in oxygen. It further remains questionable whether the grass again did not prove to be a more powerful competitor for the increase in oxygen, if any. With reference to the experiments in which aeration was prevented, it is possible that in the absence of any competition, the oxygen supplied in the water which came from aerated sources might have been sufficient to supply the needs of the trees.

The plant-food factor was checked up by growing a two-year-old tree in washed sand which contained very insignificant amounts of the nutrient elements. The tree made good normal growth for a whole year and survived during the second season without showing any of the characteristic grass effects. The experimenters concluded that the deficiency in plant food is not the factor which is responsible for the grass effects, since practically entire lack of plant food failed to produce effects similar to those produced by the grass.¹⁰

There is some question, however, whether there was an entire lack of plant food. It is possible that the amount of plant food contained in the water with which the tree was supplied was sufficient to support

⁹ Pickering, S. P., l. c.

¹⁰ Pickering, S. P., l. c.

the normal growth of a two-year-old tree for one season. It is further possible that the deficiency in total plant food is less injurious than the deficiency in one element of plant food, which might have been the case of the grassed-over trees. Would it not have been more to the point, as in the case of the moisture experiment, to have grown the two-year-old tree together with grass in the presence of an abundant supply of balanced plant food?

The principal fallacy of the experiments, however, lies in the fact that the experimenters were trying to obtain the peculiar grass effects from each of the factors singly, while these effects might have been the result of certain combinations of these factors. The peculiar grass effects as they are described by the Woburn investigators could have been ascribed with a great degree of plausibility to the combined influence of a deficiency in moisture and nitrates. This possibility is strengthened by the fact that when the soil around the trees was planted to clover, the color effects were missing.

The inability of oak trees to advance into the so-called "oak openings" (grassy tracts) which were found in the natural oak forests of Ohio and Indiana¹¹ and the antagonisms existing between butternut trees and shrubby cinquefoil, reported by Jones and Morse,¹² as well as the antagonism between peach trees and several grasses reported by Hedrick,¹³ are phenomena similar to those observed on the Woburn Fruit Farm. Toxic excreta or poisonous action in general is not the only possibility which may be offered in their explanation.

WHEAT GROWN IN ASSOCIATION WITH TREE SEEDLINGS.

Germinated wheat seedlings were grown by C. A. Jensen¹⁴ in parafined pots in association with seedlings of pine, maple, dogwood and cherry. The same number of wheat seedlings were planted in every pot. Successive crops of wheat were grown one after another for periods of two to three weeks. One of the pine seedlings died during the first crop of wheat but the pot was not discarded and received the same treatment as the other pots. Table 1 (taken intact from Bureau of Soils Bulletin No. 40) gives the relative green weights of the wheat crops.

¹¹ Schreiner, Oswald, and Reed, Howard S., Some Factors Influencing Soil Fertility, U. S. Dept. Agr., Bur. Soils Bul. No. 40, p. 37. 1907.

¹² Jones, L. R., and Morse, W. J., Ann. Rep. Vt. Agr. Expt. Sta. 16 (1903), p. 173-190. U. S. Dept. Agr., Bur. Soils Bul. No. 40, p. 17.

¹³ Hedrick, U. P. Proc. Soc. Hort. Sci., 1905, p. 72-82. U. S. Dept. Agr., Bur. Soils Bul. No. 40, p. 17.

¹⁴ Schreiner, Oswald, and Reed, Howard S., l. c., p. 18-19.

TABLE 1.—*Relative Green Weights of Wheat Crops Grown in Association With Tree Seedlings.*

Date of Harvesting.	June 29.	July 12.	Aug. 1.	Aug. 22.	Sept. 6.	Oct. 13.	Oct. 29.	Nov. 19.	Dec. 6.	Ave. First Six Crops.	Ave. Last Three Crops.
Control.....	100	100	100	100	100	100	100	100	100	100	100
Maple 1.....	76	65	86	68	67	86	92	91	96	74	93
" 2.....	44	86	75	59	71	79	90	75	109	71	91
" 3.....	21	83	72	72	79	84	81	103	92	70	92
Dogwood 1.....	92	96	76	84	71	65	85	68	115	81	89
" 2.....	86	79	63	86	75	73	84	107	88	78	93
Cherry.....	81	91	102	91	71	94	88	102	93	88	94
Tulip.....	21	106	62	77	68	100	77	109	103	75	96
Pine.....	55	69	68	52	54	80	62	83	60	63	68
Pine (dead).....	62	96	85	91	80	89	97	96	67	84	87

Table 1 shows that the wheat crops suffered from association with the tree seedlings. The depressing effects of the tree seedlings decreased toward autumn, however, as is noticeable especially when the last two columns are compared.

The authors of Bureau of Soils Bulletin No. 40 believe that the depressing effects of the tree seedlings were due to toxic excreta produced by their roots. They believe that this view is borne out by the entire behavior of the experiment. The increase of the relative yields toward autumn coincides with the period when the trees enter upon their seasonal rest and was due, according to them, to the fact that the toxic excreta were diminished, together with the decrease of the general physiological activity of the deciduous trees. The increase in the yield of the wheat crops grown in association with dogwood was less because it was the last to shed its leaves. The pot containing the living pine tree did not show any increase in the last three crops. The pot containing the dead pine gave better yields than the other tree pots but inferior to those of the controls. The authors argue that the depressing effects of the trees could not be due to depletion of plant food since "if the trees had removed sufficient plant food to starve the wheat plants in the summer period, the increased yield toward autumn would be incapable of explanation."

However, the figures presented in the table do not show any absolute increase in yield for the tree pots toward autumn. They only show that they were nearer to the yield of the controls. It is possible that the better crops of the controls had removed toward the end of the summer nearly as much plant food as the wheat crops together with the trees in the other pots and this is why there was less difference in the respective yields. Supposing that the yields actually increased toward autumn, the possibility that the depressing effect of

the trees was a plant food phenomenon is not at all excluded. The status of available plant food in the soil is dynamic in character. Plant food is continually being manufactured in the soil. The same decrease in the physiological activity of the trees during the period of rest which, according to the authors, caused a decrease in root excreta, also caused a decrease in plant food assimilation, and the increased yield of wheat in the tree pots might have been due to the lessened competition of the trees for plant food.

FAIRY RINGS.

This fanciful name is given to rings of grass in pastures or meadows which are markedly darker in color and more luxuriant in growth. In close proximity to the rings, on the outside, various fungi are always found, so that there are really two rings, a ring of grass and a ring of fungi. The diameter of the concentric rings increases every year and it is generally assumed that the smallest ring is preceded by a single point or by a small continuous area.

Schreiner and Reed¹⁵ are evidently inclined to interpret this phenomenon in the sense of De Candolle's theory which Way¹⁶ considers "by far the most scientific and most intelligent solution of the question," but which he does not accept. The phenomenon of the fairy rings would just fit in De Candolle's theory. The fungi recede because they excrete certain substances which are harmful to themselves but which are beneficial to grass. The excreta beneficial to the grass do not extend very far and therefore the luxuriant growth of grass follows the fungi in the form of an inner concentric ring.

Way ignores the fact that the fungi do not grow inside of the ring and tries to explain the luxuriant growth of the grass. The fungi, according to Way, are good collectors of the mineral plant food elements. When they die they fertilize the soil in the immediate vicinity and so cause the luxuriant growth of grass of the ring.

The authors of Bulletin No. 40 do not concern themselves with the grass, but with the fact that the fungi do not grow inside of the ring. This fact serves, according to them, as evidence in favor of autotoxic plant excreta. The failure of the fungi to grow inside of the ring can not, they say, be due to depletion in plant food, since the analysis of the soil inside and outside of the rings by Lawes and Gilbert showed too slight differences. Inside of the ring the percentage of nitrogen was 0.247 and of carbon, 2.78; outside of the

¹⁵ *l. c.*, p. 37.

¹⁶ Way, J. T., On the Fairy Rings of Pastures, etc., *Jour. Royal Agr. Soc.*, 7 (1846), pp. 549-552.

ring the percentage of nitrogen was 0.281 and of carbon 3.30. The amounts of carbon and nitrogen, however, are consistently higher outside of the ring than inside. It is possible that the difference is limited to the available organic materials on which fungi grow and, small as it is, it may be a factor which determines the growth of the fungi.

Gilbert¹⁷ interpreted the behavior of the grass and the fungi in the fairy rings entirely in the sense of the plant-food theory.

There is another objection to the use of fairy rings as evidence in favor of autotoxic plant excreta. We are hardly justified in drawing an analogy between heterotrophic and autotrophic plants, as we do know for certain that the heterotrophic plants do excrete certain organic substances such as enzymes, and that these substances are of vital significance in the economy of their nutrition. On the other hand, we do not know of any secretions by roots of autotrophic plants except carbon dioxide.

THE DIMINISHED YIELD OF SUCCEEDING CROPS.

A number of experiments with wheat in paraffined pots were conducted¹⁸ to show that the diminished yield of succeeding crops is due not to depletion of plant food but to toxic excreta produced by the previous crop. The crops were grown for periods of three or four weeks. The results show invariably that the succeeding crops were considerably lower than the first crops. They further show that the addition of fertilizers did not change to any considerable extent the proportional relations between the first and the succeeding crops, and that the addition of cowpeas and lime was more effective than the addition of mineral fertilizers.

Since young crops could not have removed sufficient plant food to account for the marked decline of the succeeding crop and since the addition of cowpeas and lime which do not furnish immediate plant food proved to be more effective than the addition of direct plant food in the form of fertilizers, the authors conclude that the depressive effect of the previous crop was due to toxic excreta. As further evidence in this connection, they consider the fact that, as shown by their experiments, the mere germination of seeds in a soil is already detrimental to the succeeding crop. Similar results were obtained by Livingston¹⁹ and his associates with soil and washed quartz sand.

¹⁷ Gilbert, J. H., Note on the Occurrence of Fairy Rings, *Jour. of Linnean Soc.*, 15 (1877), pp. 17-24.

¹⁸ Schreiner and Reed, l. c., p. 10-15.

¹⁹ Livingston, Burton Edward, Britton, J. C., and Reid, F. R., Studies on the Properties of an Unproductive Soil, U. S. Dept. Agr., Bur. Soils Bul. No. 28, 1905.

The authors of this bulletin do not make it clear whether they consider the evidence brought forward in this connection as substantiating the "toxic" interpretation of crop rotations, that is, whether they would expect different results if several crops were used in a rotation under the same conditions.

The results of these experiments would really tend to show that the depressing effects of the previous crops were not due to depletion of plant food. They do not show conclusively, however, that the inferior yields were due to toxic root excreta nor that the secretion of toxic substances might be a factor under natural field conditions. The experiments were conducted under such unnatural conditions that in many of them the depressing effects might have been due to physical deterioration of the soil. This interpretation would be in harmony with the fact that lime and cowpeas had a more decided ameliorating effect than fertilizers.

It is, however, possible that in all the results here reported the depressing effects were due to conditions associated with seed germination, since the crops in all cases were grown only for short periods.

The phenomena of seed germination are so entirely different from conditions of plant growth after the plant begins to draw its nutrition from the surrounding medium that they ought to be considered separately. The metabolic changes, both destructive and constructive, during the period of seed germination are so rapid, so many different enzymes are involved in the transformation of the products stored up in the seeds, and so much organic material is made available for the growing embryo that the products of seed germination may become harmful to plants when they accumulate, either directly or through the agency of micro-organisms which they may attract.

However, the phenomena associated with the processes of seed germination are a natural stage in the evolution of plants. Each step in the development of the plant is adapted to the corresponding stages of the seed metabolism and its normal growth is, therefore, not interfered with by the products of germination of its mother seed under natural conditions. As to the effect on the succeeding crop, the products of metabolism in the process of seed germination are of such unstable nature that they can hardly be expected to last until the next normal crop and can hardly be a factor in soil fertility under normal conditions when crops are grown to maturity.

WHEAT SEEDLINGS GROWN IN AGAR.²⁰

Segments of glass tubing about three centimeters long and having an internal diameter of 6 to 8 millimeters were fastened in a vertical position to a glass rod at intervals of 2 to 3 millimeters. The segmented tubes were placed in small jars and melted agar was poured in them till its level reached the surface of the upper segment. When the agar cooled down to 35° to 38° C., wheat seedlings were planted in the upper segments of the segmented tubes.

About 53 percent of the seedlings grew out through the openings of the segmented tubes into the surrounding agar. When the experiments were repeated with agar in which seedlings had been previously allowed to grow a smaller percentage of the roots curved into the segmented openings. When the same experiments were carried out in such a way as to eliminate the geotropic tendency of the roots by the use of a klinostat, a greater percentage of roots curved out into the openings. When fresh agar was used inside of the segmented tubes and agar in which seedlings had previously been grown outside of them, the percentage of curvatures was smaller. On the other hand, a larger percentage of curvatures was obtained when used agar was placed inside of the segmented tubes and fresh agar outside of them. Certain relationships were obtained with reference to the behavior of wheat seedlings when agar in which corn, cowpeas and oats had been grown was used outside and inside of the segmented tubes.

These facts tend to show, according to the authors, that the roots of the plants included in the experiment excrete certain substances deleterious to themselves, but less or not at all deleterious to other plants, the tendency to grow into the openings being due to the stimulus of negative chemotropism, a tendency to grow away from a harmful substance.

A closer examination of the figures presented in connection with these experiments shows so much variation between the individual experiments that we are hardly justified in considering the averages as the expression of the general tendency of the phenomena.

The curving into the openings might have been due to a cause of a physical nature, the slight tendency of the averages to behave in the expected direction being simply accidental. The growing tip, as it is generally known, exerts a considerable pressure. This perhaps forced the agar in the narrow segments into the openings and the roots were carried along with the agar, or the curving into the openings might have been due to the general spreading habits of the root system.

²⁰ Schreiner and Reed, l. c., p. 23-36.

If, however, the curving into the openings was really due to differences between the agar in the segmented tubes and the outside agar, the theory of toxic excreta is not the only possible explanation. The tendency to curve into the openings might have been due to water relations, there being more available water outside of the narrow segmented tube. This possibility becomes more plausible when we take into consideration the fact that while chemotropism in higher plants has not been definitely established, hydrotropism, or the movement toward water, is a well-known phenomenon.²¹

BEHAVIOR OF WHEAT SEEDLINGS IN WATER EXTRACTS OF SOIL.²²

The behavior of soil extracts is brought forward as evidence in favor of the existence of toxic substances in soils, regardless of their origin.

It was found that a poor soil extract yields poorer crops than distilled water. The depressing effects of the extract could not be due to lack of plant food, since it contains more of it than the distilled water. When an extract of a poor soil is diluted the yield is improved, notwithstanding the fact that the diluted extract contains less plant food. A case is reported in which the poor properties of a soil extract were transferred to its distillate, which would tend to indicate the presence of some volatile toxic substances. When a poor soil extract was used in making up a balanced solution the yields obtained were inferior to those which were obtained when the same balanced nutrient solution was made up with distilled water. The addition of substances which have no nutritive value at all, as pyrogallol, ferric hydrate and carbon black, improves greatly the crop productivity of the poor soil extract. The beneficial effects of these substances is ascribed to their power of absorbing the toxic substances present in the extracts.

All these facts would tend to show that there is something in the extracts of the poor soils which interferes with plant growth, although some of these facts do not bear necessarily on the presence of toxic bodies. It was found, for instance, that the addition of solids, as carbon black, ferric hydrate, etc., improves a good soil extract also, although not to so great an extent as it improves a poor soil extract. It is possible that the action of the added solid is absorptive, but the good soil extract also contains comparatively small amounts of toxic substances and it is, therefore, also improved by the addition of ab-

²¹ Jost, L., *Lectures on Plant Physiology*, pp. 484-485. Oxford, 1907.

²² U. S. Dept. of Agr., Bureau of Soils Bul. 28, 36, and 40.

sorbing agents. It is, however, also possible that the effect of the solid is due to some other cause, the action perhaps being on the plant rather than on the medium. It is possible, for instance, that the solids stimulate a response in plant roots just as gravity does (geotropism). If so, the different extent of the effects of the solid on the poor and good soil extract would be due either to the different properties of the extracts which affect that response differently, or to the limits of possible improvement.

As to the effects of dilution on the productive capacity of the poor soil extract, the results obtained lose much of their force as a proof in favor of the theory of soil toxicity because it has never been tried on a good soil extract. It would perhaps be found that good soil extracts would also be improved by dilution and it would then be possible to suggest some other explanation of the beneficial effect of dilution in addition to the one based on the dilution of the toxic substances.

The yields in the experiments with soil extracts were generally measured by transpiration, which is not always a reliable indicator of plant growth. The plants were grown only for periods of two to three weeks, under which condition too much significance can not be attached to differences in yield if they are not striking, as was the case in many of these experiments.

The results obtained with soil extracts in general could hardly be considered as due to the same factors which are operative in the poor soils from which they were prepared under field conditions. This is because the extracts were prepared in such a different manner for the natural soil solution (excess of solvent, shaking, etc.), and since the soil, as it was shown, has such an ameliorating influence on the poor properties of a liquid medium, even when present in very small quantities.²³

ISOLATION OF TOXIC SUBSTANCES FROM SOILS.

A number of organic compounds have been isolated by Schreiner and Shorey²⁴ from different soils and some of them have proved to be toxic to plants in water cultures, as picoline carboxylic acid and dihydroxystearic acid. With reference to picoline carboxylic acid, the authors have admitted that it was hardly a factor in soil fertility.²⁵ They think, however, that dihydroxystearic acid is directly responsible for the poor yields of the poor soils from which it has been isolated, since it shows depressing effects in water cultures even when

²³ Livingston, B. E., et al., l. c., p. 35.

²⁴ Schreiner, Oswald, and Shorey, Edmund C., *The Isolation of Harmful Organic Substances from Soils*, U. S. Dept. Agr., Bur. Soils Bul. No. 53, 1909.

²⁵ L. c., p. 47.

present in concentrations as low as 20 parts per million. It is questionable, however, as previously pointed out, whether conclusions with reference to actual field conditions can be drawn from results obtained in water cultures.

Dihydroxystearic acid has been isolated from good soils as well as from poor soils, although much more frequently from the latter than from the former. It has been admitted by Schreiner²⁶ that dihydroxystearic acid is perhaps not responsible for the low productive capacity of the poor soils from which it has been isolated and that it is possible that its presence is a result of the same conditions which render the soils poor. Furthermore, the very presence of dihydroxystearic acid in soils from which it has been isolated is not definitely established as it is possible that it is formed during the process of extraction.

SUMMARY.

It is apparent from the foregoing analysis that the evidence which is offered in favor of the theory of soil toxicity is neither direct nor conclusive. The facts on which it is based can be interpreted in a variety of ways other than the existence of toxic substances.

The question would be considered definitely settled if toxic substances isolated from a poor soil, when applied in the same quantity in which they are there present, caused a soil which does not contain them to produce a poor crop similar to that produced by the poor soil.

Indirect or circumstantial evidence is of less value in problems of soil fertility than in many other problems. So many factors known and unknown affect the soil that several interpretations of the same phenomena are frequently possible. The real significance of these phenomena may often escape us because of our lack of knowledge of the processes taking place in the soil.

(To be concluded in the September-October Journal.)

²⁶ Schreiner, O., and Lathrop, E. C., Dihydroxystearic Acid in Good and Poor Soils, Jour. Amer. Chem. Soc., 33 (1911), p. 1412-1417.

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(Continued from the July-August number.)

EXPERIMENTAL DATA.

OBJECT OF THE EXPERIMENTS.

All the laboratory work on soil toxicity dealing with introduced organic deleterious substances has been carried out in water cultures. No attempts have ever been made to determine how the toxic substances which either have been isolated or which have been thought possibly to be present in the soil would behave in actual field tests, although it could reasonably be expected that the soil through its many various agencies would greatly modify their toxic action.²⁷

In the experiments conducted by the writer during the winter of 1912-13, the principal object was to obtain some data as to how substances which were found to be toxic to plants in water cultures would affect crops grown to maturity in soil, and how these effects would be modified by lime, by each individual mineral fertilizer, and by a complete fertilizer. The work was limited to two organic toxins, cumarin and vanillin. These substances were selected because they have been used in a number of experiments with water cultures in order to demonstrate the behavior of organic toxins, and because they could be easily obtained in sufficient quantities in a pure state.

Experiments with water cultures and quartz cultures were conducted parallel with the field experiments.

EXPERIMENTS WITH SOIL.

The experiments with soil were conducted in 3-gallon pots in the greenhouse. The soil used was Dunkirk clay loam from the experimental plots of the Cornell University Agricultural Experiment Station. Ten kilograms of soil were weighed out in each pot. The pots were watered as frequently as was necessary, once a week at the beginning of the experiment when there was very little transpiration and two to three times a week toward the period of maturity.

²⁷ Since this paper was prepared, a bulletin by Schreiner and Skinner (Harmful Effects of Aldehydes in Soils, U. S. Dept. Agr. Bul. No. 108, 1914) has appeared which gives the results of field plat tests with toxic substances.

Only distilled water was used. The moisture content of the soil at the time of watering was 30 percent on the dry basis. The pots were kept under a mulch of white quartz sand. Thirty-six wheat seeds were sown in each pot, the stand afterward being thinned to 12 plants per pot.

The toxins were added in parts per million and were figured on the basis of the highest total moisture content of the soil at the time of watering. The concentrations used were 200, 100, and 10 parts per million for cumarin, and 1,000, 500 and 10 parts per million for vanillin. The highest concentrations used were twice the killing concentrations in water cultures.

The experiments consisted of six series: (1) Without additional treatment, (2) with lime, (3) with nitrogen, (4) with phosphoric acid, (5) with potash and (6) with a complete fertilizer. Nitrogen was added as sodium nitrate, phosphoric acid as disodium phosphate, potassium as potassium chloride and lime as calcium hydroxide. Nitrogen, phosphoric acid and lime were added on the basis of 400 pounds per acre, and potassium on the basis of 200 pounds per acre. Only chemically pure materials were used. Each series consisted of fourteen pots, two for each concentration of the toxins used and two control pots. In the tables which follow, the figures reported are the averages from the duplicate pots in each case.

The toxins, the fertilizers and the lime were added in the dissolved state only. The lime was added as lime water which had been titrated against a standard acid. The addition of toxins was repeated three times, each time to the extent of a full equivalent of the total moisture.

Effect on Germination.

About three weeks after planting, new seedlings ceased to appear above ground. Before thinning, the seedlings were counted in order to see whether the different treatments had any effect on the germinating power of the seeds. The percentages of germination and the relative germination as compared with the germination in the control pots in each series are given in Table 2.

The only conclusion which would seem to be justified from this table is that none of the different treatments had any effect on germination. The percentages are very irregular, the differences between the duplicates being larger than between the individual treatments. The variations seem to be mere fluctuations and seem to be due to general conditions affecting germination, as heredity and general environmental factors, and not to any single factor arising from a particular treatment.

TABLE 2.—*Effect of Different Concentrations of Cumarin and Vanillin on the Germination of Wheat, as Shown by the Percentage of Germination and the Ratio of Each to the Control.*

Toxin.	P.p.m.	No Treat- ment.		CaO.		N.		P ₂ O ₅ .		K ₂ O.		Complete Fertilizer.	
		Ratio.		Ratio.		Ratio.		Ratio.		Ratio.		Ratio.	
Cumarin ..	200	53	76.8	67	80.7	81	117.4	64	79	69	107.8	69	88.5
" ..	100	64	92.8	86	103.6	78	113	81	100	67	104.7	69	88.5
" ..	10	75	109	75	90.4	75	108.7	67	82.7	69	107.8	75	96.2
Control	69	100	83	100	69	100	67	100	64	100	78	100	
Vanillin...	1,000	67	97.1	83	100	69	100	72	88.9	75	117.2	86	110.3
" ..	500	61	88.4	83	100	72	104.3	72	88.9	75	117.2	78	100
" ..	10	75	109	72	86.7	67	97.1	78	96.3	78	121.9	81	103.8

Effect on Yield.

The plants in the pots were grown to maturity. Observations taken during the period of growth did not lead to any definite conclusions. It seemed from time to time that the stand in the cumarin pots of the two higher concentrations was inferior to that of the control pots in some of the series, especially in the nonfertilized and in the limed series. However, no abnormalities in the appearance of the plants in these pots were observed. They looked normally green and as healthy as the plants in the other pots.

In Table 3 the weights of the water-free substance for the grain and straw and the total yield are given. The table gives the average weights for the duplicates and the proportional values of the averages with reference to the control pots in each series, the yields of which are taken as 100.

In the weights of the grain shown in Table 3 there is a certain regularity in the nonfertilized and in the limed series. The highest concentrations give inferior yields; the lower concentrations give yields either equal to or slightly higher than the control pots. In the nitrogen series the regularity is preserved with reference to the cumarin pots, while in the vanillin pots the yields are arranged in the reverse order, the differences, however, being very small. The phosphoric acid series preserves the regularity except for the pots which received the highest concentration of vanillin. These latter gave an abnormally high yield as compared with the remaining pots of the same series. The potassium series varies very little and not in the expected direction. The complete fertilizer series is very regular, the variations being gradual and pronounced as in the first two series.

The weights of the straw are on the whole less regular than those of the grain. The nonfertilized series follows the regular order

completely. The limed series also varies in the expected direction except in the case of the second concentration of cumarin. The remaining series do not show any regularity in their variations.

TABLE 3.—Weights (Water-free Substance) of Grain, Straw and Total Weight Obtained from Pot Cultures of Wheat Variouslly Fertilized and Treated with Different Concentrations of Cumarin and Vanillin, with the Ratio of Each to the Control Taken as 100.

WEIGHTS OF GRAIN.

Toxin.	P.p.m.	No Treat- ment.		CaO.		N.		P ₂ O ₅ .		K ₂ O.		Complete Fertilizer.	
		Weight.	Ratio.	Weight.	Ratio.	Weight.	Ratio.	Weight.	Ratio.	Weight.	Ratio.	Weight.	Ratio.
		Gm.		Gm.		Gm.		Gm.		Gm.		Gm.	
Cumarin ..	200	4.21	64.8	6.12	74.5	14.9	85.1	4.96	80.5	6.34	100.8	10.9	63
" ..	100	5.08	78.1	7.55	92.0	18.3	104.6	6.42	104.2	6.61	105.1	13.8	79.8
" ..	10	6.75	103.9	7.69	93.7	18.9	108	7.16	116.2	6.58	104.6	16.3	94.2
Control		6.50	100	8.21	100	17.5	100	6.16	100	6.29	100	17.3	100
Vanillin ..	1,000	5.79	89.1	5.66	68.9	16.8	96	9.96	161	6.93	110.2	15.2	87.9
" ..	500	6.72	103.4	6.48	78.9	16.3	93.1	6.14	99.7	6.37	101.3	15	86.7
" ..	10	7.47	114.9	8.28	100.9	16.1	92	6.55	106.3	6.58	104.6	15.6	90.2

WEIGHTS OF STRAW.

Cumarin ..	200	23.4	85.4	25.9	92.5	54.7	102	25.7	101.6	22.4	104.7	40.0	95.2
" ..	100	25.9	94.5	29.5	105.3	55.7	103.9	27.6	109.1	21.1	98.6	45.9	109.3
" ..	10	28	102.2	27.5	98.2	53.2	99.3	27.4	108.3	24.5	114.5	44.7	106.4
Control		27.4	100	28	100	53.6	100	25.3	100	21.4	100	42	100
Vanillin ..	1,000	24.6	89.8	22.9	81.8	48.9	91.2	34.8	137.6	24.9	116.4	43.9	104.5
" ..	500	27.1	98.9	24.7	88.2	50.3	93.8	24.2	95.6	20.3	94.9	47.5	113.1
" ..	10	28	102.2	28.5	101.8	52.5	97.9	23.2	91.7	19	88.8	46.4	110.5

TOTAL WEIGHTS.

Cumarin ..	200	27.6	81.4	32.1	88.6	69.7	96.7	30.7	97.4	28.7	103.6	50.8	85.8
" ..	100	31	91.4	37.1	102.5	74.1	102.8	34	107.9	28.7	103.6	54.8	92.2
" ..	10	34.8	102.7	35.2	97.2	72.1	100	34.5	109.5	31.1	112.3	61	102.7
Control		33.9	100	36.2	100	72.1	100	31.5	100	27.7	100	59.4	100
Vanillin ..	1,000	30.4	89.7	28.6	79	65.7	91.1	44.7	141.9	31.8	114.8	59.1	99.5
" ..	500	33.8	99.7	31.2	86.2	66.6	92.4	30.4	96.5	26.7	96.4	62.5	105.2
" ..	10	35.4	104.4	36.5	100.8	68.7	95.3	29.7	94.3	25.5	92.1	62	104.3

The total yields of the grain and straw naturally occupy a place between the weights of each separately with reference to the tendency to show a regular trend in any direction. The nonfertilized and the limed series again prove to be the most regular. The complete fertilizer series comes next, being quite regular in the cumarin pots. The nitrogen series shows a tendency to regularity, being more regular in the vanillin half. The potassium series again proved to be the least regular. On the whole, the variations in the total yield are not very sharp.

The general impression produced by examination of Table 3 would be that the highest concentrations of the cumarin and vanillin caused a slight depression in yield, especially in the yield of grain, and that the depression is more pronounced and more regular in the nonfertilized, in the limed, and in the complete fertilizer series. The addition of the individual fertilizers seemed to have a disturbing influence on the tendency to follow the regular effects of the toxins used.* No conclusion can be drawn as to whether it was accidental (which is not improbable in view of the fact that the variations in general were not very sharp) or whether it was due to the influence of the fertilizer. Neither is it possible to draw any conclusions with reference to the effects of the individual fertilizers in the absence of distinct variations since there was only one series for each fertilizer. The complete fertilizer did not seem to modify the effects of the cumarin and vanillin.

Effect on the Nitrogen Content.

The nitrogen content may serve sometimes as an indication of the presence of certain factors which influence the general yield of a crop. If a depression in yield is caused by some accidental factor, the tendency is in the direction of a relatively higher nitrogen content. The reason for this phenomenon might be due either to the fact that the interfering factor does not affect the production of nitrates in the soil, or that it does not affect the assimilative power of the plant for nitrogen. It was thought, therefore, that the nitrogen content of the crops might throw some light on the nature of the influence exerted by the cumarin and vanillin treatment. Table 4 gives the percentages of nitrogen in the grain and in the straw and the ratios of the different treatments to the control, taking the percentage of nitrogen in the controls as 100.

Examining these tables, we find that the percentages of nitrogen in the straw fluctuate too irregularly to allow of any generalizations. We find, for instance, that in the nonfertilized and the limed series, which proved to be the most regular ones with reference to the weight of water-free substance the highest concentrations of cumarin and vanillin gave approximately the same percentages as the controls. In the remaining series, the concentration of 200 parts per million of cumarin gave somewhat higher percentages than the controls. The other concentrations of cumarin, as well as all the concentrations of vanillin, do not follow any regular order.

TABLE 4.—*Nitrogen Content of Wheat Grain and of Straw Grown in Pot Cultures Variouslly Fertilized and Treated with Different Concentrations of Vanillin and Cumarin, with the Ratio of Each to the Control Taken as 100.*

WHEAT GRAIN.

Toxin.	P.p.m.	No Treat- ment.		CaO.		N.		P ₂ O ₅ .		K ₂ O.		Complete Fertilizer.	
		Nitrogen.	Ratio.	Nitrogen.	Ratio.	Nitrogen.	Ratio.	Nitrogen.	Ratio.	Nitrogen.	Ratio.	Nitrogen.	Ratio.
Cumarin ..	200	1.72	107.5	1.91	107.9	2.27	113.5	2.15	114.4	2.06	106.7	2.15	108.6
" ..	100	1.75	109.4	1.76	99.4	2.01	100.5	2.00	106.4	1.95	101	2.09	105.5
" ..	10	1.81	113.1	1.58	89.3	2.07	103.5	1.96	104.3	1.89	97.9	2.10	106.1
Control ..		1.60	100	1.77	100	2.00	100	1.88	100	1.93	100	1.98	100
Vanillin...	1,000	1.76	110	1.89	106.8	1.89	94.5	1.97	104.8	1.89	97.9	2.03	102.5
" ..	500	1.82	113.7	1.68	94.9	2.01	100.5	1.98	105.3	1.93	100	2.06	104
" ..	10	1.68	105	1.81	102.3	2.04	102	1.93	102.7	1.97	102.1	2.13	107.6

WHEAT STRAW.

Cumarin ..	200	0.39	102.6	0.33	103.1	0.46	135.3	0.45	118.4	0.39	114.7	0.35	112.9
" ..	100	.31	81.6	.34	106.2	.41	120.6	.33	86.8	.32	94.1	.35	112.9
" ..	10	.31	81.6	.38	118.8	.38	111.8	.34	89.5	.35	102.9	.35	112.9
Control ..		.38	100	.32	100	.34	100	.38	100	.34	100	.31	100
Vanillin...	1,000	.42	110.5	.32	100	.36	105.9	.36	94.7	.30	88.2	.29	93.6
" ..	500	.41	107.9	.30	93.7	.40	117.6	.36	94.7	.32	94.1	.39	125.8
" ..	10	.30	78.9	.33	103.1	.43	126.5	.39	102.6	.37	108.8	.42	135.5

The percentages of nitrogen in the grain exhibit a regularly borne out consistency with reference to the concentration of 200 parts per million of cumarin, as they are consistently higher than those of the controls in all the series. With reference to the remaining concentrations of cumarin and those of vanillin, the consistency varies with the series, and does not, on the whole, allow any generalizations as in the case of the straw.

The depression in yield of grain, which was most pronounced in the case of the concentration of 200 parts per million of cumarin, would seem to be accompanied by a relatively higher nitrogen content which is characteristic of the presence of some factor interfering with plant growth. The question is whether the effect of the interfering factor was directly on the plant, causing some morphological derangement or interfering with its physiological functions, or whether the effect was on the medium in which it grew.

The appearance of the plants in the pots to which the toxic substance had been added was perfectly normal, as stated above. The roots were found to permeate the soil in every direction and did not show any inferior development as compared with the controls. The

depression in yield was not evidently due to lack of nitrogen, but the possibility of its being due to a relative deficiency in some of the other available elements of plant food, actual or physiological, caused by the interfering factor, is not excluded. It is also possible that the interfering factor affected the physical and biological conditions of the soil.

Effect on Nitrates.

In order to obtain some idea of the effects of the toxins used on the biological activity of the soil, the soil in the pots was analyzed for nitrates.

The pots could not be handled immediately after the crops were harvested, but were held at a low moisture content and were analyzed, a complete series at a time, so that each series could be compared only with its own control pots. During the sampling, the soil was removed from the pots, pulverized, and thoroughly mixed. After the first sampling, the soil was returned to the pots, kept at about 25 percent of moisture for a time, and analyzed again. This was analogous to incubation, as the pots were kept in the greenhouse and were all subject to the same variations in temperature, which (the weather at that time of the year being constant) were limited only to the difference of the day and night temperature. Again, one series was handled at a time, so that each series was incubated for a different length of time.

The nitrates in parts per million are given in Tables 5 and 6. In Table 5 the averages of the duplicate pots are compared with the averages of the control pots in each series, the latter being taken as 100. In Table 6 the increase in nitrates for the period of incubation was calculated and the average increase of the duplicate pots is compared with the average increase of the control pots taken as 100.

TABLE 5.—*Nitrates in Pot Cultures Variously Fertilized and Treated with Different Concentrations of Cumarin and Vanillin, as Determined before Incubation, with the Ratio of Each to the Control Taken as 100.*

Toxin.	P.p.m.	No Treat- ment.		CaO.		N.		P ₂ O ₅ .		K ₂ O.		Complete Fertilizer.	
		NO ₃ , P.p.m.	Ratio.	NO ₃ , P.p.m.	Ratio.	NO ₃ , P.p.m.	Ratio.	NO ₃ , P.p.m.	Ratio.	NO ₃ , P.p.m.	Ratio.	NO ₃ , P.p.m.	Ratio.
Cumarin ..	200	15.6	82.5	33.5	97.1	20.1	57.8	37.3	119.2	36.6	106.1	52.2	64.3
" ..	100	14.2	75.1	31.8	92.2	22.9	65.9	44.8	143.1	36.6	106.1	52.8	65
" ..	10	16	84.7	32.3	93.6	31.6	90.8	36.5	116.6	36.7	106.4	51.6	63.5
Control ..		18.9	100	34.5	100	34.8	100	31.3	100	34.5	100	81.2	100
Vanillin ..	1,000	9.4	49.7	19.8	57.4	23.2	66.7	60.6	193.6	31	89.9	52.2	64.3
" ..	500	11.2	59.3	24.7	71.6	38.3	110	35.0	113.7	33.7	97.7	43.6	53.7
" ..	10	15.2	80.4	26	75.4	29.9	85.9	32.5	103.8	21.5	62.3	55.9	68.8

TABLE 6.—*Increase of Nitrates after Incubation from Pot Cultures Variously Fertilized and Treated with Different Concentrations of Cumarin and Vanillin, with the Ratio to the Control Taken as 100.*

Toxin.	P.p.m.	No Treat- ment.		CaO.		N.		P ₂ O ₅ .		K ₂ O.		Comp. Fert.
		Incr., P.p.m.	Ratio.	Incr., P.p.m.	Ratio.	Incr., P.p.m.	Ratio.	Incr., P.p.m.	Ratio.	Incr., P.p.m.	Ratio.	Incr., P.p.m.
Cumarin..	200	42.9	129.6	23.9	85.4	21.6	73	29.9	198	14.6	69.2	17.4
" ..	100	41.7	126	23.5	83.9	26.4	89.2	31	208.3	9.5	48.1	9.9
" ..	10	34.3	103.6	27.5	98.2	33.7	113.8	29.7	196.7	21.5	101.9	16.3
Control.....		33.1	100	28	100	29.6	100	15.1	100	21.1	100	Loss
Vanillin...	1,000	24.3	73.4	19.5	69.6	31.9	107.8	27.5	182.1	14.9	70.6	14.3
" ..	500	28.2	84.6	19.1	68.2	22.2	75	23.4	155	11.2	53.1	15.5
" ..	10	51.7	156.2	24.6	87.9	26.5	89.5	22.9	151.6	9.9	46.9	25.4

As is seen in Table 5, the nitrates in four series are consistently higher in the control pots than in the pots to which the toxins had been added. The phosphoric acid series goes in the opposite direction and the potassium series is only partially consistent.

Table 6, presenting the data for the incubation period, shows that in the toxin-treated pots of the lime, the nitrogen, and the potassium series the increase in nitrates is lower than in the control pots. The phosphoric acid goes the other way as in the period preceding incubation. In the complete fertilizer series, the control pots showed a reduction in nitrates instead of an increase, so that there is no standard of comparison. In the nonfertilized series, the increase in the control pots was larger than in the vanillin pots, but smaller than in the cumarin pots. This might have been due to the fact that this series was incubated for the longest period of time and the cumarin was completely decomposed. As will be seen later, there are indications that the effects of cumarin are more subject to amendment through decomposition by the soil agencies than are the effects of vanillin.

The general impression produced by the figures representing the results of the analysis for nitrates, would be that the addition of the toxins used seemed to interfere with nitrification.

The behavior of the cumarin and the vanillin with reference to their effects on nitrification would seem to be analogous to the behavior of soluble organic matter in general. König, Hasenbäumer and Glenk²⁸ found that the addition of glucose invariably inhibited nitrification. The inhibiting effect of soluble organic matter on

²⁸ König, J., Hasenbaumer, J., and Glenk, K., *Über die Anwendung der Dialyse, etc., Landw. Vers. Stat., 79-80 (1913), p. 491-534.*

nitrification might be either directly on the nitrifying organism or on the soil factors affecting their activity. Another possibility is that the soluble organic matter stimulates the growth of other bacteria which compete with the nitrifying organisms for the means of subsistence. König and his associates invariably found higher bacterial numbers as a result of the addition of glucose.

Effect on Conductivity.

The same authors found that the addition of glucose reduced the conductivity of the soil. The explanation suggested by them is that the glucose acts protectively toward the electrolytes of the soil, thus interfering with the movements of the ions. In discussing the depression in yield, which under certain conditions is produced by the addition of sugar, the authors suggest that the electrical conductivity or the movement of the ions is in itself a factor in soil fertility. It was, therefore, interesting to see how the cumarin and vanillin, which produced a slight depression in yield practically similar to that produced by the addition of glucose, would affect the conductivity of the soil in the experimental pots.

Fifty grams of soil were thoroughly mixed with 50 percent of distilled water on the dry basis, and the resistance measured by a Wheatstone bridge. Tables 7 and 8 show the resistance in ohms calculated at 60° F. before and after incubation.

The results are not entirely consistent, but nevertheless a general inspection of the tables gives the impression that the soil in the pots to which cumarin and vanillin had been added showed a somewhat higher conductivity as compared with the control pots, for the period following incubation.

TABLE 7.—*Effect on Conductivity of Various Fertilizers and Treatments with Different Concentrations of Cumarin and Vanillin, as Shown by the Resistance in Ohms Calculated at 60° F. before Incubation, with the Ratio of Each to the Control.*

Toxin.	P.p.m.	No Treat- ment.		CaO.		N.		P ₂ O ₅ .		K ₂ O.		Complete Fertilizer.	
		Resist- ance.	Ratio.	Resist- ance.	Ratio.	Resist- ance.	Ratio.	Resist- ance.	Ratio.	Resist- ance.	Ratio.	Resist- ance.	Ratio.
Cumarin	200	2,174	104.2	1,970	96.7	2,137	112.7	1,779	82.3	1,609	115.2	1,774	99.7
"	100	2,229	106.8	2,003	99.6	2,250	118.7	1,720	79.6	1,700	121.7	1,081	94.5
"	10	2,369	113.5	2,181	107.1	1,996	105.3	1,951	90.3	1,701	121.8	1,702	95.7
Control		2,087	100	2,038	100	1,896	100	2,161	100	1,397	100	1,779	100
Vanillin	1,000	2,416	115.8	2,324	114	1,875	98.9	1,711	79.1	1,579	113	1,682	94.5
"	500	2,195	105.2	2,146	120.1	1,550	81.8	1,981	91.7	1,337	95.7	1,711	96.3
"	10	1,979	94.8	2,484	121.9	1,675	88.3	1,951	90.3	1,944	139.1	1,423	80

TABLE 8.—*Effect on Conductivity of Various Fertilizers and Treatments with Different Concentrations of Cumarin and Vanillin, as Shown by the Resistance in Ohms after Incubation Calculated at 60° F., with the Ratio of Each to the Control.*

Toxin.	P.p.m.	No Treatment.		CaO.		N.		P ₂ O ₅ .		K ₂ O.		Complete Fertilizer.	
		Resistance.	Ratio.	Resistance.	Ratio.	Resistance.	Ratio.	Resistance.	Ratio.	Resistance.	Ratio.	Resistance.	Ratio.
Cumarin	200	1,680	90	1,515	93.2	1,550	110	1,499	86.8	1,356	102	1,642	103.2
"	100	1,867	100	1,580	97	1,700	120	1,430	82.8	1,455	109	1,488	93.5
"	10	1,929	103	1,633	105	1,487	105	1,653	95.7	1,381	103.8	1,540	96.8
Control		1,867	100	1,625	100	1,412	100	1,727	100	1,330	100	1,591	100
Vanillin	1,000	1,736	93	1,817	112	1,475	104.5	1,430	82.8	1,307	98.3	1,540	96.8
"	500	1,744	95	1,732	107	1,525	108	1,628	94.3	1,233	92.7	1,565	98.4
"	10	1,649	88.3	1,694	104	1,600	113	1,801	104.3	1,529	115	1,283	80.6

This, however, is not directly contradictory to the results obtained by König and his associates, since we have introduced the crop factor, while their results were obtained without growing any crop in the soil experimented with, and since the measurements in these experiments were made a comparatively much longer time after the organic substances were added.

The soil in the cumarin and the vanillin pots seemed to show after incubation a higher conductivity in spite of the fact that the respective treatments apparently reduced their nitrate content. It is possible that the plants withdrew from the soil, in the presence of the toxins used, less of the other electrolytes. This might have been due either to the fact that the toxins interfered directly with the absorption of these electrolytes by the plant, or that they stimulated the growth of microorganisms which held the electrolytes tied up in their tissues at the time of active plant growth.

That organic substances which prove to be toxic to higher plants in water cultures may be favorable to the growth of microorganisms, was shown by the fact that a solution containing 200 parts per million of cumarin showed to the naked eye an abundant growth of molds and fungi when allowed to stand for some time. The same was true with dihydroxystearic acid which had been isolated from a soil in Tompkins County, New York.

It is remarkable that König and his associates failed to see the possibility of any connection between the higher bacterial numbers resulting from the addition of sugar and the other phenomena resulting from the same treatment, as depression in yield, lowering of conductivity, and the reduction in the nitrate content. Such a con-

nection would not be improbable according to the works of Stoklasa,²⁹ Severin³⁰ and Duschetschkin.³¹

EXPERIMENTS WITH WATER CULTURES.

Effect on Germination.

Wheat seeds were allowed to germinate on filter paper in petri dishes. Fifty seeds were placed in each dish to which 15 c.c. of solutions of the respective concentrations of cumarin and vanillin were added. Fifteen c.c. of distilled water were added to the control petri dishes. The test was run in duplicate. Observations were taken after six and ten days. Table 9 shows the results obtained after six days.

TABLE 9.—*Effect of Different Concentrations of Cumarin and Vanillin on the Germination of Wheat in Water Cultures.*

Toxin.	P.p.m.	Average Germina- tion.	Percent.	Description of Seedlings.
Cumarin....	200	0	0	
".....	100			
".....	10	22	44	Normally developed seedlings.
Control.....		22	44	Better than in the previous case.
Vanillin.....	1,000	5	10	Very weak.
".....	500	9	18	Better than in previous case. Some poor but normal seedlings.
".....	10	26	52	Vigorous.

Most of the seeds which are recorded as not germinated in reality made slight efforts to germinate, but evidently the seedlings were killed in the earliest stage of embryonic development.

The second observations did not reveal any changes with reference to the cumarin treatment, except that the seeds were all overgrown with molds and fungi. The vanillin petri dishes, however, showed considerable improvement, especially those which received 500 parts per million. In these the percentage of germination and the percentage of normally developed seedlings increased considerably (germination, 32 percent; normally developed seedlings, 26 percent).

It is thus seen that cumarin had a more injurious effect on germination than vanillin. It is also seen that the deleterious effect of

²⁹ Stoklasa, Julius, *Biochemischer Kreislauf des Phosphat-ions im Boden*, Centbl. Bakt., 29, (II), 1911, p. 385-519.

³⁰ Severin, S. A., *Changes of Phosphoric Acid in the Soil, etc.*, Centbl. Bakt., 28, (II), 1910, p. 561-580.

³¹ Duschetschkin, A., *Biological Absorption of Phosphoric Acid*, Jour. Exp. Agr. (Russia), 12, p. 650-666.

the vanillin decreased with the time, in spite of the fact that the respective solutions became more concentrated due to evaporation.

Effect of Cumarin on the Growth of Seedlings Grown in Nutrient Solution of Various Concentrations.

Tumblers of 250 c.c. capacity were used. They were covered with paraffined paper in which small holes were made with a pointed glass rod. The roots of germinated seedlings were carefully introduced into the tumblers filled with nutrient solution, through the holes of the paper covers, so that the attached seeds remained rested on the upper side of the paper. Four seedlings were planted in each tumbler.

The nutrient solution used was of the following composition:

Calcium nitrate	2.7 gm. per liter.
Monopotassium phosphate	1.5 gm. per liter.
Magnesium sulphate	0.6 gm. per liter.
Potassium chloride	0.75 gm. per liter.
Ferric sulphate	0.05 gm. per liter.

This nutrient solution was used in full strength and also diluted 3 and 10 times respectively. The concentrations of cumarin were 200, 100, and 10 parts per million. The experiment was run in triplicate. The seedlings were grown for two weeks. The dry weights determined collectively for each set of triplicates are given in Table 10.

TABLE 10.—*Dry Weights of Wheat Seedlings Grown for Two Weeks in Different Concentrations of Cumarin.*

Cumarin.	Concentration of Nutrient Solution.	Dry Weights.	Relative Weights.
<i>p.p.m.</i>		Grams.	
200	1 : 10	Killed after 7 days	
100	1 : 10	" " " "	
10	1 : 10	0.2562	82
Control	1 : 10	.3120	100
200	1 : 3	Killed after 7 days	
100	1 : 3	" " " "	
10	1 : 3	.5160	76
Control	1 : 3	.6820	100
200	1 : 1	Killed after 7 days	
100	1 : 1	" " " "	
10	1 : 1	.4630	60
Control	1 : 1	.7696	100

As seen from Table 10, the depressing effect of 10 parts per million of cumarin is greater in the nutrient solution of the higher concentration. It is evident, however, that the increased nutrient content did not increase the deleterious action of the toxin, since the absolute yields are higher in the higher concentrations of the nutrient solu-

tion. The deleterious effects of the toxin were more pronounced in the case of the higher concentrations of the nutrient solution, probably because the yields in general were higher with these concentrations.

It is clear, however, that the higher concentrations of the nutrient did not reduce the toxicity of the cumarin. The ameliorating effect of phosphoric acid on cumarin reported by Schreiner and Skinner³² is evidently not antagonistic in character, nor is it evidently due to the fact that phosphoric acid increases the resisting power of the plant to the action of the toxin, since an increased concentration of this substance did not have the same effect in a balanced solution.

It is possible that the results obtained by Schreiner and Skinner were due to the residual effect of the source of phosphoric acid after the latter was used. It is also possible that the presence of cumarin does not interfere with the absorption of phosphoric acid, while it does interfere with the absorption of the other nutrient elements, and therefore the difference in yield between the controls and the cumarin cultures are more pronounced in the case of distilled water and a balanced nutrient solution than in the case of a solution of phosphoric acid.

Effects of Small Quantities of Soil on the Behavior of Cumarin and Vanillin.

The methods used were in the main similar to those in the previous experiment. The nutrient solution used was the one given above, diluted four times. The experiment was run in triplicate, and consisted of two series which differed only in the fact that each tumbler of the second series received 2 grams of field soil. Both series were run simultaneously, and under exactly the same conditions. The

TABLE II.—*Dry Weights of Wheat Seedlings Grown for Two Weeks in Water Cultures Containing Different Concentrations of Cumarin and Vanillin, with and without the Addition of Small Quantities of Soil.*

Toxin,	Concentration,	Solution Without Soil.		Solution Plus 2 Grams of Soil.	
		Average Weight,	Ratio.	Average Weight,	Ratio.
	<i>p.p.m.</i>	<i>Grams.</i>		<i>Grams.</i>	
Cumarin.....	200	Killed after 7 days		0.1625	99
".....	100	" " " "		.1708	104
".....	10	0.1473	77	.1874	115
Control.....		.1901	100	.1631	100
Vanillin.....	1,000	Killed after 7 days		Killed after 7 days	
".....	500	" " " "		" " " "	
".....	10	.1817	92	.1552	95

³² Schreiner, Oswald, and Skinner, J. J., Organic Compounds and Fertilizer Action, U. S. Dept. of Agr., Bur. Soils Bul. No. 77. 1911.

seedlings were grown for two weeks. The average weights of the water-free substance from the triplicate cultures are given in Table II.

As seen from Table II, the toxic effects of the cumarin were completely destroyed by the addition of a very small quantity of soil. As the table shows, the cumarin tumblers of the second series gave higher yields than the controls, but it is safer to ignore this fact since we can not base too much on small differences in yields obtained from seedlings grown for two weeks. It is clear, however, that the toxic effects of the cumarin were entirely overcome. This was also shown by the appearance of the seedlings, especially by the appearance of the roots, which were perfectly healthy and very much branched in the concentration of 200 parts per million of cumarin. The comparison with the similarly treated cultures which had not received any soil is so striking that the ameliorating effect of the soil on the action of cumarin under the conditions of this experiment is beyond any doubt. On the behavior of the seedlings in the vanillin solutions, on the other hand, the addition of soil did not have any effect whatsoever.

The effect produced by the soil in the case of cumarin was probably due not to adsorption, since the quantity of soil was so small, but to decomposition. The difference in the effects of the soil on cumarin and vanillin may be due either to the fact that vanillin is not as readily decomposed by the soil organisms as cumarin, or to the fact that the products of decomposition of vanillin are just as toxic as vanillin itself, while the decomposition products of cumarin are not toxic.

This experiment would suggest that the depressing effect of cumarin on the yields in the experiments with soil might be due to different causes than those operative in water cultures.

EXPERIMENT WITH QUARTZ CULTURES.

The experiment was carried out in half-gallon pots. White quartz sand thoroughly washed with hydrochloric acid was used. Two kilograms of quartz were used per pot. The nutrient solution given above in which enough cumarin and vanillin were dissolved to make up the usual respective concentrations, was added to each pot to the extent of 25 percent of the weight of the quartz. The pots were kept at 25 percent of moisture, and were watered with distilled water. Nutrient solution was added from time to time. The addition of the toxins when repeated was in solutions of the respective concentrations and in equivalents of the total moisture. Sixteen wheat seeds were planted in each pot. The germinated seedlings were thinned out to 5 per pot. The experiment was run in duplicate.

Effects on Germination.

After the seedlings ceased to appear above ground, they were counted, and the percentage of germination and the relative values, taking the controls as 100, were calculated. The results are given in Table 12.

TABLE 12.—*Effect of Different Concentrations of Cumarin and Vanillin on the Germination of Wheat in Quartz Cultures.*

Toxin.	Concentration.	Average Germination.	Percentage.	Ratio to Control.
	<i>p.p.m.</i>			
Cumarin.....	200	None		
".....	100	None		
".....	10	11	69	92
Control.....		12	75	100
Vanillin.....	1,000	13	81	108
".....	500	10	62	84
".....	10	14	87	116

As seen from Table 12, cumarin had the same effects on germination in quartz as in a liquid medium. The seeds in the pots of the two highest concentrations, when dug out, had the same appearance as in the petri dishes—slightly swelled and having made a very slight effort to germinate.

Vanillin did not have any effect at all on germination in quartz cultures. The higher results than in the control pots as well as the lower results obtained in these cultures are to be regarded as mere fluctuations.

Effect on Growth.

After the thinned-out seedlings had been grown for about seven weeks, the tops were harvested and the weights of the water-free substance determined. The results are given in Table 13.

TABLE 13.—*Dry Weights of Wheat Seedlings Grown in Quartz Cultures Treated with Different Concentrations of Cumarin and Vanillin.*

Toxin.	Concentration.	Number of Equivalents Added.	Average Weight.	Ratio to Control.
	<i>p.p.m.</i>		<i>Grams.</i>	
Cumarin.....	200	1	Did not come up above quartz	
".....	100	1	Did not come up above quartz	
".....	10	2	1.6	94
Control.....			1.7	100
Vanillin.....	1,000	3	1.25	74
".....	500	3	1.65	97
".....	10	3	1.60	94

As seen from Table 13, the cumarin behaved in quartz cultures in the same way as in water cultures without the addition of soil. The vanillin behaved approximately as in the soil.

GENERAL DISCUSSION.

Since vanillin behaved the same way in quartz sand with a comparatively low adsorptive power as in a clay soil with a comparatively high adsorptive power, the ameliorating effects of the quartz and of the soil on vanillin were probably not due to adsorption. Since, however, the soil organisms added to the water cultures with the small quantities of soil did not have any effect on the action of vanillin, the ameliorating effect of soil and quartz on this toxin was probably not due to decomposition. In all probability, vanillin is only toxic when applied in a liquid medium which envelops the roots completely in a continuous layer. This would seem to be borne out by the experiment on the effects of cumarin and vanillin on germination in a liquid medium; on longer standing the inhibiting effect of vanillin on germination decreased, in spite of the fact that the solution became more concentrated, due to evaporation.

The case is entirely different with reference to cumarin. A small quantity of soil added to water cultures containing 200 parts per million of cumarin, which is double the killing concentration, completely destroyed the injurious effects of this toxin. The distribution of the toxic solution in films on the surface of the quartz grains did not have any ameliorating effect at all on the action of cumarin. Evidently the ameliorating effect of the soil on this toxin was due to its decomposing power. Adsorption is in all probability excluded, since the small quantities of soil added to the water cultures could not have adsorbed the comparatively large quantities of the toxin in the higher concentrations. Evidently, the ameliorating effect of the soil on cumarin and vanillin demonstrated in the experiments with soil was due to different causes.

The experiments did not show clearly that the depressing effect of cumarin and vanillin on the yield of the crops grown in soil, which was more pronounced with reference to the yield of grain, was due to the same causes which are operative in water cultures. On the other hand, there are some indications that the effect of the toxins is due to different causes in the soil than in water cultures. The appearance of the crops in the pots which received the highest concentrations of the toxins was perfectly healthy. No inhibiting effect on the growth of the roots has been observed. A small quantity of soil added to the water cultures which contained cumarin entirely

destroyed its toxic effects. All these considerations would tend to suggest that the depression in yield observed was not a case of toxicity, which implies a certain morphological derangement or certain changes in the composition and constitution of the plant substance which interfere with the normal physiological functions of the plant.

Depressions in yields were obtained with glucose, which substance one would hardly consider as a toxin.³³

The depressing effects of cumarin and vanillin on the crops grown in soil might be due to the general effect of soluble, non-nutrient organic matter.

Soluble organic matter may affect the microflora of the soil, stimulating the growth of harmful organisms or the growth of microorganisms in general which would tend to tie up available plant food, or inhibiting the growth of useful bacteria. Thus, the results of these experiments would tend to show that the highest concentrations of cumarin and vanillin had a depressing effect on nitrification.

The presence of soluble organic matter may affect to a certain extent the physical condition of the soil, forming protective films on the soil particles and thus interfering with granulation.

Soluble organic matter which is not used by the plant may interfere, as a foreign substance present in the soil solution, with the absorption by the plant of the necessary elements of plant food.

It might be added that these experiments were conducted under conditions which entirely excluded drainage, and that the frequent watering of the pots tended to compact the soil very much. It is possible that under proper conditions of drainage and cultivation, the results would be different.

On the whole, it might be said that these experiments would hardly lend much support to the assumption that the presence in the soil of organic substances toxic in water cultures is a factor of considerable importance under field conditions, when the other factors of plant growth are normally good.

SUMMARY.

1. The evidence offered in favor of the theory of soil toxicity is not sufficient to establish the fact that the roots of higher plants excrete substances harmful to themselves or to other plants. Neither is the evidence sufficient to establish the presence in the soil of organic substances harmful to plants under normal field conditions.

³³ König, Hasenbäumer, und Glenk, l. c.

2. The concentrations of 600 parts per million of cumarin and of 3,000 parts per million of vanillin, figured on the basis of the total moisture content of the soil, depressed to some extent the yield of wheat grown to maturity in pots. There are indications, however, that the effect was rather on the soil than on the plant. .

3. The addition of small quantities of soil to water cultures entirely destroyed the toxic effects of cumarin, while it did not affect the action of vanillin. It is possible that vanillin is less readily decomposed by the microorganisms of the soil than cumarin, or that the decomposition products of vanillin are as toxic in water cultures as vanillin itself.

4. In quartz cultures, cumarin has proved to be as toxic as in water cultures, while vanillin behaved approximately the same way as in the soil. Vanillin is evidently toxic only in a liquid medium when it is applied in mass, but not when it is distributed as films over quartz grains or soil particles.

5. The ameliorating effect of phosphoric acid on the action of cumarin reported in Bulletin 77 of the Bureau of Soils would not seem to be due to its antagonistic behavior with reference to that toxin, since it did not behave in the same way in a balanced solution. The ameliorating effects reported might be due either to the residual effects of the base after the phosphate radical was used up, or to the fact that cumarin does not interfere with the absorption by the plant of phosphoric acid while it does interfere with the absorption of the other food elements.

6. The behavior of toxic substances is so different in the soil than in water cultures, that one is hardly justified in drawing conclusions from results obtained with water cultures as to what might take place under actual field conditions.

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